

Figure 4 is a photograph of an autoradiogram of product of linear ligation amplification of  $\beta^S$  substrates on sickle cell (lanes c and g) and normal beta globin (lanes d and h) oligonucleotide templates.

Figure 5A is a photograph of autoradiograms of reaction products from six rounds of exponential ligation amplification of substrates designed to be complementary to the sickle cell gene sequence using sickle cell (HB23S') and normal (HB23A') oligonucleotide template.

Figure 5B is a logarithmic plot of fold of amplification versus number of exponential ligation amplification rounds performed.

Figure 6 is a photograph of an autoradiogram of the product of from 10-14 rounds of exponential ligation amplification using templates from plasmids containing normal (lanes g-i) and sickle cell (lanes d-f) beta globin gene using substrates complementary to the sickle cell beta globin gene sequence (Figure 2) and normal beta globin gene sequence (Figure 1).

Figures 7A and 7B are photographs of autoradiograms resulting from amplification of human genomic DNA using  $\beta$  thalassemia (lanes a, b), sickle cell (lanes c, d), and normal  $\beta$  globin (lanes e, f) DNA template. Figure 7A resulted from ligating  $\beta^S$  substrates; Figure 7B resulted from ligating  $\beta^A$  substrates.

Figure 8 is a photograph of an autoradiogram of a linear amplification resulting from the use of a pair of  $\beta^S$  substrates and human genomic DNA template from  $\beta$ -thalassemia (lane a), sickle cell (lane b), and normal  $\beta$  globin (lane c). Lanes d-f are controls.

Figure 9A is a photograph of an ethidium bromide stained agarose gel containing PCR amplified 294 base pair fragments of alleles at the human beta globin gene.

Figures 9B(a) and 9B(b) are photographs of autoradiograms of PCR products from the Figure 9A gel transferred to nylon membranes and hybridized with radiolabeled HB19S (panel a) and radiolabeled HB19A (panel b) probes.

Figure 9C is a photograph of an autoradiogram of ligation amplification products using PCR enriched sequences as templates.

#### DEFINITIONS

Point mutation--a single nucleotide substitution in the sequence of a gene.

Target sequence--a DNA sequence that may or may not include a point mutation.

Template--a single stranded DNA sequence that includes a target sequence.

Substrate--an oligonucleotide sequence complementary to a region of a template which, when annealed to said template, can be ligated to an adjacently annealed second substrate.

Template dependent ligation--ligation of substrates annealed to a complementary template.

Template independent ligation--any ligation of oligonucleotides which is not a function of the degree of substrate-template complementarity, e.g., blunt end ligation of DNA sequences resulting from the substrate hybridization.

#### DETAILED DESCRIPTION OF THE INVENTION

##### 1. Template Target Sequences

Templates useful in the invention may be obtained from various sources, e.g., any natural or synthetic DNA or RNA. The invention has particular applicability to templates derived

from cloned DNA and genomic DNA or RNA derived from human blood or tissue. Such templates may include one or a plurality of the same or different sequences which may be amplified simultaneously. Single stranded templates are obtained from double stranded DNA by known denaturing techniques, for example, by heating at temperatures from about 80° to 105°C for about 1 to 10 minutes or by enzymes. See, e.g., Wallace, et al. (1980).

Target sequences include, for example, the causative point mutations for genetic diseases such as sickle cell anemia,  $\alpha$ - and  $\beta$ -thalassemia, phenylketonuria, hemophilia, or  $\alpha_1$ -antitrypsin deficiency.

## 2. Substrates

Substrates useful in the invention are oligonucleotide sequences complementary to sequences which immediately flank each side of a variant nucleotide in a target sequence. Substrates may be synthesized by known methods using commercially available synthesizers such as Applied Biosystems 380 B automated DNA synthesizers. See Wu, et al. (1989). Substrate length depends on various factors including the temperature of the reaction, pH, and complexity of the target sequence. Substrate sequences of from about 4 to about 100 nucleotides are generally useful particularly at higher reaction temperatures. In the preferred practice of the invention, the substrates are sequences of from about 8 to about 25 nucleotides.

Typical substrate pairs useful for the template-dependent ligation by T4 DNA ligase are set forth in Table 1.